

1

SEQUENCE LISTING

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<120> photoproteins with enhanced bioluminescence and assays using the same

<130> 1489EUR

<160> 22

<170> PatentIn version 3.1

<210> 1

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<212> PRT

<213> Clytia gregaria

<400> 1

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Asp Asn Pro Lys Trp Val Asn Arg His Lys Phe Met Phe Asn Phe Leu
20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

2

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
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Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<213> Unknown

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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Ser Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

3

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<213> Unknown

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<223> Clytin mutant: mutClyK4

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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

4

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Cys Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
180 185 190

Tyr Gly Asn Phe Val Pro
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<212> PRT

<213> Unknown

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35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

5

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

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Tyr Gly Asp Phe Val Pro
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<213> Unknown

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<223> Clytin mutant: 1H7 mutant

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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

6

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Arg Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Val Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Ile Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: 1C12 mutant

<400> 6

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Asp Asn Pro Lys Trp Val Asn Arg His Lys Phe Met Phe Asn Phe Leu
20 25 30

7

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Phe Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Asn Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Leu Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Arg Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
180 185 190

Tyr Gly Asn Phe Val Pro
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<210> 7

<211> 198

<212> PRT

<213> Unknown

<220>

<223> Clytin mutant: 25N03b mutant

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20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Cys Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
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Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<223> Clytin mutant: 3C12 mutant

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Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Val Cys Ala Lys Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Arg Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<212> PRT

<213> Unknown

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<223> Clytin mutant: 6H22 mutant

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Asp Ile Asn Gly Asp Gly Lys Val Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Arg Leu Gly Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Asp Ala Val Glu Ala Phe Phe Lys Lys Ile Gly Met
65 70 75 80

Asp Tyr Gly Lys Glu Val Glu Phe Pro Ala Phe Val Asp Gly Trp Lys
85 90 95

Glu Leu Ala Asn Tyr Asp Leu Lys Leu Trp Ser Gln Asn Lys Lys Ser
100 105 110

Leu Ile Arg Asp Trp Gly Glu Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
165 170 175

Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
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<210> 10

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<223> Clytin mutant: 12mutCly

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20 25 30

Asp Ile Asn Gly Asp Gly Lys Ile Thr Leu Asp Glu Ile Val Ser Lys
35 40 45

Ala Ser Asp Asp Ile Cys Ala Lys Leu Glu Ala Thr Pro Glu Gln Thr
50 55 60

Lys Arg His Gln Val Cys Val Glu Ala Phe Phe Arg Gly Cys Gly Met
65 70 75 80

Glu Tyr Gly Lys Glu Ile Ala Phe Pro Gln Phe Leu Asp Gly Trp Lys
85 90 95

Gln Leu Ala Thr Ser Glu Leu Lys Lys Trp Ala Arg Asn Glu Pro Thr
100 105 110

Leu Ile Arg Glu Trp Gly Asp Ala Val Phe Asp Ile Phe Asp Lys Asp
115 120 125

Gly Ser Gly Ser Ile Ser Leu Asp Glu Trp Lys Ala Tyr Gly Arg Ile
130 135 140

Ser Gly Ile Cys Ser Ser Asp Glu Asp Ala Glu Lys Thr Phe Lys His
145 150 155 160

Cys Asp Leu Asp Asn Ser Gly Lys Leu Asp Val Asp Glu Met Thr Arg
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Gln His Leu Gly Phe Trp Tyr Thr Leu Asp Pro Asn Ala Asp Gly Leu
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Tyr Gly Asn Phe Val Pro
195

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<211> 600

<212> DNA

<213> Unknown

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<223> Clytin mutant: mutClyK1_dna

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cccgagcaga ccaagagaca ccaggacgoc gtggaggcct tcttcaagaa gatcggcatg 240
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tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctggac 540
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<210> 12

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: mutClyK4_dna

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accctggacg agatcgtgag caaggccagc gacgacatct gcgccaaagt gggcgccacc 180
cccgagcaga ccaagagaca ccaggacgoc gtggaggcct tcttcaagaa gatcggcatg 240
gactacggca aggaggtgga gttccccgcc ttcgtggacg gctggaagga gctggccaac 300
tacgacctga agctgtggag ccagaacaag aagagcctca tcagggaactg gggcgaggcc 360

13

gtgttcgaca ttttcgacaa ggacggcagc ggctgcatca gcctggatga gtggaaggcc 420
tacggcagaa tcagcggcat ctgcagcagc gacgaggacg ccgaaaagac cttcaagcac 480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
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<210> 13

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1F10 mutant_dna

<400> 13

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acctggacg agatcgtgag cagggccagc gacgacatct gcgccaaagt gggcgccacc 180
cccgagcaga ccaagagaca ccaggacgcc gtggaggcct tttcaagaa gatcgcatg 240
gactacggca aggaggtgga gttcccgcc ttctggacg gctggaagga gctggccaac 300
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tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
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<210> 14

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1H7 mutant_dna

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accttgagc	agatcgtgag	caaggccagc	gacgacatct	gcgccaagct	ggcgccacc	180
cccgagcaga	ccaagagaca	ccgggacgcc	gtggaggcct	tcttcaagaa	gatcggcatg	240
gactacggca	aggaggtgga	gttccccgtc	ttcgtggacg	gctggaagga	gctggccaac	300
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gtgtttgaca	tcttcgacaa	ggacggcagc	ggcagcatta	gcctggatga	gtggaaggcc	420
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tgcgacctgg	acaacagcgg	caagctggac	gtggacgaga	tgaccagaca	gcacctgggc	540
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<210> 15

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 1C12 mutant_dna

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accttgagc	agatcgtgag	caaggccagc	gacgacatct	gcgccaagct	ggcgccacc	180
cccgagcaga	ccaagagaca	ccaggacgcc	gtggaggcct	tcttcaagaa	gatcggcatg	240
gacttcggca	aggaggtgga	gttccccgcc	ttcgtggacg	gctggaagga	gctggccaac	300
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gtgttcgaca	tcctcgacaa	ggacggcagc	ggcagcatca	gcctggatga	gtggaaggcc	420
tacggcagaa	tcagcggcat	ctgcagaagc	gacgaggacg	ccgaaaagac	cttcaagcac	480
tgcgacctgg	acaacagcgg	caagctggac	gtggacgaga	tgaccagaca	gcacctgggc	540
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<210> 16

<211> 600

<212> DNA

15

<213> Unknown

<220>

<223> Clytin mutant: 25N03b mutant_dna

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accctggacg	agatcgtgag	caaggccagc	gacgacatct	gcgccaaagct	gggcgccacc	180
cccagacaga	cgaagagaca	ccaggacgcc	gtggaggcct	tcttcaagaa	gatcggcatg	240
gactacggca	aggaggtgga	gttccccgcc	ttcgtggacg	gctggaagga	gctggccaac	300
tacgacctga	agctgtggag	ccagaacaag	aagagcctca	tcagggaactg	gggcgaggcc	360
gtgttcgaca	tcttcgacaa	ggacggcagc	ggcagcatca	gcctggatga	gtggaaggcc	420
tactgcagaa	tcagcggcat	ctgcagcagc	gacgaggacg	ccgaaaagac	cttcaagcac	480
tgcgacctgg	acaacagcgg	caagctggac	gtggacgaga	tgaccagaca	gcacctgggc	540
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<210> 17

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 3C12 mutant_dna

<400> 17

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accctggacg	agatcgtgag	caaggccagc	gacgacgtct	gcgccaaagct	gggcgccacc	180
cccagacaga	ccaagagaca	ccaggacgcc	gtggaggcct	tcttcaagaa	gatcggcatg	240
gactacggca	aggaggtgga	gttccccgcc	ttcgtggacg	gctggaagga	gctggccaac	300
tacgacctga	agctgtggag	ccaaaacaag	aagagcctca	tcagggaactg	gggcgaggcc	360
gtgttcgaca	tcttcgacaa	ggacggcagc	ggcagcatca	gcctggacga	gtggaaggcc	420
tacggcagaa	tcagcggcat	ctgcagaagc	gacgaggacg	ccgaaaagac	cttcaagcac	480

16

tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccagaca gcacctgggc 540
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<210> 18

<211> 600

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 6H22 mutant_dna

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<211> 597

<212> DNA

<213> Unknown

<220>

<223> Clytin mutant: 12mutCly_dna

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17

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gtgttcgaca ttttcgacaa ggacggcagc ggcagcatct ctctggacga gtggaaggcc 420
tacggccgga tcagcggcat ctgcagcagc gacgaggacg ccgagaaaac cttcaagcac 480
tgcgacctgg acaacagcgg caagctggac gtggacgaga tgaccggca gcacctgggc 540
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<210> 20

<211> 32

<212> DNA

<213> Unknown

<220>

<223> synthetic primer

<400> 20

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32

<210> 21

<211> 33

<212> DNA

<213> Unknown

<220>

<223> synthetic primer

<400> 21

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<210> 22

<211> 33

<212> DNA

<213> Unknown

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<220>

<223> synthetic primer

<400> 22

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33

<210> 23

<211> 27

<212> DNA

<213> Unknown

<220>

<223> synthetic primer

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